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Long-term effects of boron supplementation on reproductive characteristics and bone mechanical properties in gilts^{1,2,3,4}

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ABSTRACT: An experiment was conducted to determine long-term effects of dietary boron (B) on reproductive and bone characteristics in gilts. Weanling gilts (n = 50) were allotted to 10 pens based on weaning weight and litter origin. Pens were randomly assigned to receive one of two dietary treatments that consisted of a basal diet low in B (control) and the basal diet supplemented with 5 mg of B/kg diet as sodium borate. Gilts remained on their respective experimental diets throughout the nursery phase, growing-finishing phase, sexual maturity, breeding, gestation, and lactation. The day of first observed standing estrus was defined as puberty, and each pubertal gilt was bred via AI at the second observed standing estrus. Eight randomly selected gilts per treatment were slaughtered at d 35 of gestation for the assessment of embryonic and reproductive characteristics, bone characteristics, and tissue B concentrations. The remaining pregnant gilts (con-

trol, n = 11; 5 mg supplemental B/kg diet, n = 10) farrowed, and litter characteristics at farrowing and weaning were determined. Age at puberty was not affected ($P = 0.72$) by B, and neither were the number of corpora lutea on the ovaries ($P = 0.44$) or the total number of embryos ($P = 0.95$) at d 35 of gestation. Boron supplementation increased ($P = 0.05$) pig weaning weight and tended ($P = 0.11$) to increase pig birth weight; however, no other litter characteristics were affected ($P \geq 0.12$) by B. Extrinsic and intrinsic strength measures of bone were increased ($P \leq 0.09$) by B. Fat-free bone ash percentage and bone mineral concentrations were not affected ($P \geq 0.19$) by dietary B. Supplemental B increased ($P \leq 0.06$) the B concentrations of the muscle, liver, and reproductive tissues. Serum osteocalcin concentrations tended ($P = 0.13$) to be increased by dietary B, which may be related to increased bone turnover in B-supplemented gilts. Results indicate that B may have beneficial effects upon reproductive and bone characteristics.

Key Words: Bones, Boron, Pigs, Reproduction

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Introduction

Warrington (1923) reported that boron (B) was required by the broad bean for growth, and now it is accepted that B is an essential element for growth and reproduction of all vascular plants (Lovatt and Dugger, 1984). The study of B essentiality in animal and human nutrition has increased, due to the report that B partially corrected leg abnormalities in cholecalciferol-deficient chicks (Hunt and Nielsen, 1981). Research has demonstrated that B may have a physiological role in both animal and human nutrition. Bone mechanics and bone ash percentage have been increased by B (Elliot and Edwards, 1992; Armstrong et al., 2000). In addition, B has been linked to the metabolism of macrominerals (Brown et al., 1989; Hegsted et al., 1991), energy metabolism (Hunt, 1997a), and the immune system (Hunt and Idso, 1999; Armstrong et al., 2001).

Boron may also play a role in reproduction and development. Low-B culture conditions have resulted in ab-

normal development and increased malformations in *Xenopus* embryos (Fort et al., 1998; 1999). A deficiency of B resulted in impaired embryonic development in rats (Lanoue et al., 1999), and B stimulated growth and increased survivability of trout and zebrafish embryos, respectively (Eckhert, 1998; Rowe and Eckhert, 1999). The present study was conducted to evaluate the long-term effects of B on reproductive characteristics and bone mechanical properties of gilts.

Materials and Methods

Animal Care and Feeding

The experimental protocols used in this study were approved by the North Carolina State University Institutional Animal Care and Use Committee.

Gilts ($n = 50$) from a terminal cross (PIC 326 \times PIC 326) were weaned at 18 to 22 d of age with an average initial weight of 6.9 kg. The gilts were allotted to 10 pens based on weaning weight and litter origin, and gilts were housed five per pen. Pens were randomly assigned to receive one of two dietary treatments, and each treatment was replicated five times. Treatments consisted of a low B basal diet (control; treatment 1) and the basal diet supplemented with 5 mg B/kg diet (treatment 2). Dietary protein sources were chosen to minimize the intrinsic dietary B concentration (Hunt, 1997b). Boron was supplemented as sodium borate decahydrate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10 \text{H}_2\text{O}$; 11.34% B; Sigma Chemical Co., St. Louis, MO). Gilts remained on their respective dietary treatment throughout the nursery phase, growing-finishing phase, sexual maturity, breeding, gestation, and lactation. Boron content of the gestation and lactation basal diets was 2.1 and 2.9 mg B/kg diet, respectively. Animal care and experimental diets for the nursery and growing-finishing phases have been reported elsewhere (Armstrong et al., 2001). The analyzed B content of the nursery, growing, and finishing diets was 0.98, 2.1, and 2.2 mg B/kg diet, respectively (Armstrong et al., 2001).

When the animals reached 175 d of age, they were moved from a finishing floor to concrete slatted pens (1.8- \times 3.1-m) in a curtain-sided, environmentally controlled building. Beginning at d 175, gilts received 1.8 kg/d of a gestation diet (Table 1) according to their preassigned experimental treatment. Gilts were checked once a day for signs of estrus by evaluating the standing reflex response to back pressure and by inspection of the vulva. A mature boar was present in the pen and had contact with the gilts for 5 to 10 min. Day of the first observed standing estrus was recorded, and this was designated as the age at puberty. Gilts were checked for estrus until they reached 250 d of age, after which time a gilt was removed from the study if she had not exhibited external signs of a behavioral estrus.

All gilts that exhibited a behavioral estrus by 250 d of age were artificially inseminated at the second

Table 1. Composition of the basal diets (as-fed basis) for gestating and lactating gilts

Item	Gestation ^a	Lactation ^b
	%	
Ground corn	84.7	78.0
Dried skim milk blend	7.0	10.0
Menhaden fish meal	6.3	10.0
Limestone	0.82	0.81
Monobasic sodium phosphate	0.43	0.44
Salt	0.50	0.50
Vitamin-trace mineral premix ^c	0.25	0.25

^aCalculated to contain 3368 kcal/kg ME, 13.7% CP, 0.72% lysine, 0.75% Ca, 0.61% P, and analyzed to contain 2.1 mg B/kg diet.

^bCalculated to contain 3375 kcal/kg ME, 16.4% CP, 0.97% lysine, 0.97% Ca, 0.73% P, and analyzed to contain 2.9 mg B/kg diet.

^cSupplied per kilogram of diet: vitamin A, 11,000 IU; vitamin D, 220 IU; vitamin E, 33 IU; menadione, 3.3 mg; riboflavin, 6.6 mg; D-pantothenic acid, 26.4 mg; niacin, 88 mg; vitamin B₁₂, 33.0 μ g; thiamin, 2.2 mg; pyridoxine, 3.3 mg; folic acid, 0.66 mg; D-biotin, 0.11 mg; Fe, 30 mg as ferrous sulfate; Zn, 30 mg as zinc sulfate; Mn, 15 mg as manganous sulfate; Cu, 5 mg as cupric sulfate; I, 1.3 mg as ethylenediamine dihydroiodide; and Se, 0.3 mg as sodium selenite.

observed standing estrus with 4×10^9 spermatozoa (total volume = 60 mL) once each day of estrus. All gilts were inseminated with semen collected from a single boar (Newsham UL). Following breeding, gilts were moved to individual crates, where they remained throughout gestation. On d 30 post-breeding, pregnancy status of the bred gilts was assessed using real-time ultrasonography (Ultrascan 45; Alliance Medical USA, Inc., Smithville, MO) with a 3.5 MHz sector probe.

Pregnant gilts were individually housed in gestation crates until approximately d 107 of gestation. During gestation, gilts were fed 2.0 kg/d of their respective diet. Diets were offered in meal form and formulated to meet or exceed the requirements of gestating sows (NRC, 1998). All pregnant gilts were moved to farrowing facilities at approximately d 107 of gestation. Animals were thoroughly hand-washed immediately prior to entering the farrowing room. Gilts were housed in individual farrowing crates from approximately d 107 of gestation to the end of lactation (21-d lactation). Once animals entered the farrowing crate, gilts were fed 1.4 kg of feed two times per day until parturition. Following parturition, the feed intake of gilts was increased each day until all gilts were consuming 3.7 kg of feed twice a day. The lactation basal diet is shown in Table 1.

Reproductive Characteristics

On d 35 of gestation, eight randomly selected gilts per treatment were sacrificed for the assessment of embryonic data. Immediately prior to death, venous blood samples were obtained for the determination of serum osteocalcin concentrations. The entire reproductive tract was excised upon death of the gilt and examined. Total number of corpora lutea on the ovaries, total number of embryos, number of live embryos, and number of dead embryos were counted to calculate fertilization

rate and embryonic viability. Live embryos were defined as being healthy and intact with clear amniotic fluid. Dead embryos were defined as being necrotic or partially resorbed with dark amniotic fluid. Fertilization rate was determined by dividing the total number of embryos by the total number of corpora lutea and by multiplying this quotient by 100. Embryonic viability was determined by dividing the number of live embryos by the total number of embryos and by multiplying this quotient by 100. Samples of the ovary, oviduct, embryo, endometrium, semitendinosus muscle, and liver were harvested and frozen at -70°C until analyses for concentration of B, calcium (Ca), and phosphorus (P). Boron content of tissues and basal diets, and Ca and P contents of tissues were determined using inductively coupled argon plasma atomic emission spectrophotometry (Varian Liberty II; Varian, Inc., Sugarland, TX). Tissues and basal diets were wet-ashed prior to analysis according to the procedures of Hunt (1997c).

Gilts pregnant at d 30 post-breeding that were not used for the assessment of embryonic data (treatment 1, $n = 11$; treatment 2, $n = 10$) were allowed to complete gestation and farrowing. At farrowing, total number of pigs born, number of pigs born alive, number of stillborn pigs, and frequency of mummified fetuses were recorded for each gilt. In addition, each live pig within a litter was weighed within 24 h post-farrowing to determine pig birth weight and litter birth weight. Within 24 h post-farrowing, all newborn pigs had needle teeth clipped, tails docked, and received an injection of an iron dextran solution. Following parturition, all litters were standardized within treatments on the basis of pig size. At weaning, the total number of pigs weaned per sow was recorded, and each pig within a litter was weighed to determine pig weaning weight and litter weaning weight.

Bone Mechanical and Chemical Properties

Regardless of pregnancy diagnosis, right femurs were harvested from each of the eight randomly selected gilts that were slaughtered at d 35 of gestation. The femurs were cleaned of adhering muscle and connective tissue, and frozen at -20°C until assessment of bone mechanical properties. Prior to mechanical testing, all femurs were removed from the freezer, thawed, and mechanical testing was immediately conducted at 23°C .

Bone mechanical properties were determined from the load-deformation curve generated from a three-point bending test (ASAE Standard S459, 1992) using an Instron Universal Testing Instrument (Model 1122; Instron, Canton, MA) and the TestWorks 4 software package (version 4.02; MTS System Corporation, Eden Prairie, MN). The crosshead speed was constant at 10 mm per min. The full-scale load of the load cell was 5,000 newtons (N), and none of the bones failed or fractured at or below 5,000 N. Therefore, it was not possible to calculate mechanical data at the failure point. As a result, all measures of mechanical properties of the

femur were determined at the yield point on the load-deformation curve. The yield point is the point on the load-deformation curve after which plastic or permanent damage occurs to the bone. Prior to the yield point, the slope of the line is linear, and the bone is undergoing elastic deformation (Crenshaw et al., 1981b). If an applied force is removed during elastic deformation, a bone will return to its original shape, and no permanent damage will have occurred to the bone (Turner and Burr, 1993). If the applied force were to be removed from the bone during elastic deformation, the bone would not return to its original shape (Crenshaw et al., 1981a,b). Mechanical properties determined at the yield point were: bending moment (kN-mm), stress ($\text{MPa} = \text{N}/\text{mm}^2$), modulus of elasticity (MPa), and deformation (mm). These mechanical properties of bone have been previously described by Crenshaw et al. (1981a,b) and Armstrong et al. (2000).

Immediately after testing for mechanical properties, 3- to 4-mm cross-sections were obtained from the shaft of the femur at the point of loading. Trabecular bone and marrow were carefully and completely removed from the cross-section, and the outside and inside diameters of the bone (both parallel and perpendicular to the applied force) were measured for the calculation of cross-sectional area moment of inertia (mm^4 ; Crenshaw et al., 1981a). Moment of inertia is a measure of the area distribution around the axis of center load in the direction of the applied force (Turner and Burr, 1993).

Cross-sections (3- to 4-mm) were obtained from the femurs immediately after testing, which were independent of the cross-sections used for the determination of moment of inertia. The fat-free ash percentage and the lipid percentage of the femur cross-sections were determined after removing the marrow as previously described (Armstrong et al., 2000). Bone Ca, magnesium (Mg), copper (Cu), and zinc (Zn) concentrations were determined by flame atomic absorption spectrophotometry (AA-6701F; Shimadzu, Kyoto, Japan). Bone ash P concentrations were determined colorimetrically by reaction with ammonium molybdate using a commercial kit (Procedure no. 670; Sigma Diagnostics, St. Louis, MO).

Serum Characteristics

Blood samples obtained at d 35 of gestation were transported back to the laboratory where serum was obtained by centrifugation at $1670 \times g$ for 30 min at 5°C and subsequently stored at -70°C . Serum was analyzed for osteocalcin concentrations using a commercial ELISA kit (NovoCalcin; Metra Biosystems, Mountain View, CA) after a 1:20 dilution of serum samples.

Statistical Analyses

Statistical analyses of data were performed using SAS (SAS Inst. Inc., Cary, NC). Age at puberty was analyzed using the GLM procedure with the statistical

Table 2. Effect of boron supplementation to a low boron diet on number of gilts exhibiting a standing estrus by 250 day of age, average age at puberty, and fertility characteristics

Item	Supplemental B, mg/kg		P-value
	0	5	
Age at puberty, d ^a	201.2 ± 4.5	203.6 ± 4.8	0.72
Range, d	181–246	178–249	
Number cycled by 250 d	22/24 (91.7%)	19/24 (79.2%)	0.22
Number bred at second standing estrus	22/22 (100%)	19/19 (100%)	1.00
Number farrowed or pregnant at d 30 post-breeding	18/22 (81.2%)	17/19 (89.5%)	0.49

^aThe mean for age at puberty represents 24 observations per treatment.

model containing dietary treatment. Because the age range at puberty was large, animals were not bred at the same time. As a result, assessment of the total number of corpora lutea, total number of embryos, number of live and dead embryos, fertilization rate, embryonic viability, tissue mineral concentrations, and bone characteristics at d 35 of gestation was conducted at two different time points. Time of collection was either August (treatment 1, $n = 5$; treatment 2, $n = 3$) or September (treatment 1, $n = 3$; treatment 2, $n = 5$) of 1999. Therefore, these data were analyzed using GLM procedures with treatment, time of collection, and appropriate interactions included in the model. The categorical or enumeration data (number of gilts cycled by d 250, number of gilts bred at second estrus, and number of gilts farrowed or pregnant at d 30 post-breeding) were analyzed via chi-square tests (Steel et al., 1997). The statistical model for litter characteristics contained dietary treatment, farrowing group, and appropriate interactions. Farrowing group was based on individual gilt breeding age and consisted of two groups. Group one farrowed between October 28 and November 29, 1999 (treatment 1, $n = 9$; treatment 2, $n = 5$), and group two farrowed between December 22, 1999 and January 21, 2000 (treatment 1, $n = 2$; treatment 2, $n = 5$). The frequency of mummified fetuses was analyzed using a categorical analysis (chi-square test), because of a lack of a normal distribution and an equal error variance for these data. All data were analyzed for outliers (Steel et al., 1997) using the UNIVARIATE procedure of SAS. If an observation was classified as an outlier, it was removed from the data set prior to analysis. A P -value of < 0.10 was considered as statistically significant, and a P -value between 0.10 and 0.15 was considered as a tendency for a treatment effect.

Results and Discussion

Dietary B did not affect ($P = 0.72$) the age at puberty in gilts (Table 2). The age at puberty in this experiment was approximately 200 d, which is comparable to other published reports (Flowers et al., 1989; Sterning et al.,

1998). Dietary B did not affect ($P \geq 0.22$) the number of gilts that cycled by 250 d of age, number of gilts bred at the second observed standing estrus, or number of gilts farrowed or pregnant by d 30 post-breeding (Table 2).

Reproductive characteristics were assessed at d 35 of gestation in eight randomly selected gilts per treatment. One gilt in each treatment that was bred at the second observed standing estrus was not pregnant at d 35 of gestation. Therefore, data collected from only seven gilts per treatment were used in these analyses. At d 35 of gestation, B had no effect ($P \geq 0.18$) on number of visible corpora lutea, total number of embryos, and the classification of the embryos as either alive or dead (Table 3). As a result, B did not alter ($P \geq 0.40$) fertilization rate or embryonic mortality. However, B-supplemented gilts had a numerically decreased ($P = 0.18$) number of dead embryos. This dependent variable was not statistically affected by supplemental B, and this could be attributed to the small sample size. Boron supplementation to low B culture conditions stimulated embryonic trout growth (Eckhert, 1998), and increased survivability of zebrafish embryos (Eckhert and Rowe, 1999; Rowe and Eckhert, 1999). In addition, *Xenopus*

Table 3. Effect of boron supplementation on litter characteristics and fertilization rate in gilts at day 35 of gestation^a

Item	Supplemental B, mg/kg		SEM	P-value
	0	5		
Number of corpora lutea	15.7	14.8	0.72	0.44
Total number of embryos	12.7	12.5	1.2	0.95
Number of live embryos	10.8	12.0	1.2	0.48
Number of dead embryos	1.9	0.5	0.67	0.18
Fertilization rate, % ^b	80.9	85.0	7.0	0.69
Percent viable embryos ^c	88.0	94.2	5.0	0.40

^aEach means represents seven observations per treatment.

^bFertilization rate = (total number of embryos/number of corpora lutea) \times 100.

^cPercent viable embryos = (number of live embryos/total number of embryos) \times 100.

Table 4. Litter characteristics at farrowing and weaning of gilts consuming low-boron diets without or with supplemental boron

Item	Supplemental B, mg/kg		SEM	P-value
	0 ^a	5 ^b		
Total number born	10.0	9.6	0.98	0.79
Number born alive	9.3	8.8	0.99	0.75
Number of stillbirths	0.17	0.70	0.23	0.12
Frequency of mummified fetuses	3/11 (27.3%)	1/10 (10.0%)	—	0.31
Average pig birth weight, kg	1.10	1.24	0.059	0.11
Litter birth weight, kg	9.95	10.50	0.97	0.70
Number of pigs weaned	6.5	7.4	0.55	0.27
Average pig weaning weight, kg	4.81	5.61	0.27	0.05
Litter weaning weight, kg	32.31	40.33	3.90	0.17

^aEach mean represents 11 observations per treatment.^bEach mean represents 10 observations per treatment.

laevis embryos derived from low-B parents had an increase in necrosis, mortality, and malformation frequencies (Fort et al., 1999). Boron-deficient conditions impaired and delayed embryonic development in mice (Lanoue et al., 1999).

At parturition, B supplementation did not affect ($P \geq 0.75$) the total number of pigs born or the number of pigs born alive (Table 4). Boron supplementation tended ($P = 0.12$) to increase the number of stillborn pigs at parturition; however, B supplementation did not affect ($P = 0.31$) the frequency of mummified fetuses at parturition (Table 4).

The average pig birth weight tended to be increased ($P = 0.11$) by B supplementation (Table 4). Average weaning weight was increased ($P = 0.05$) by B supplementation (Table 4). A litter of pigs from a B-supplemented gilt had an average pig weaning weight of 3.19 kg, and was classified as an outlier and removed from the data set, according to statistical analysis. The range after removal of the outlier was 4.21 to 6.59 kg. Previous work in our laboratory showed that B supplementation increased ADG in growing-finishing (Armstrong et al., 2001) and nursery pigs (Armstrong and Spears, 2001). Boron did not affect ($P \geq 0.17$) the number of pigs weaned or total litter weights at farrowing or at weaning (Table 4). However, numerically, litter weaning

weights averaged 8.0 kg heavier ($P = 0.17$) for B-supplemented gilts.

Measures of bone mechanical properties were increased ($P \leq 0.09$) by supplemental B (Table 5). The total applied force at the yield point and the bending moment at the yield point of the femur were increased ($P = 0.09$) in B-supplemented gilts. Intrinsic strength or yield stress of the femur was increased ($P = 0.07$) by B supplementation. Technically, bone strength is an intrinsic property of bone. Therefore, bone strength is independent of bone size or shape, and bone stress may be the best representation of bone strength (Turner and Burr, 1993). These current data agree with previous work, which has indicated that B supplementation increased the bending moment of the femur in barrows fed a semi-purified diet (Armstrong et al., 2000). This is an extrinsic measure of bone strength, because bending moment varies with bone size and shape (Turner and Burr, 1993). Other studies also indicate that supplemental B can increase the mechanical properties of bones in poultry (Rossi et al., 1993; Wilson and Ruszler, 1997; 1998) and rats (Chapin et al., 1997; 1998). Boron supplementation did not affect ($P \geq 0.33$) the modulus of elasticity of the femur at the yield point, or the cross-sectional area moment of inertia. However, femurs from

Table 5. Effect of boron supplementation on femur weight and mechanical properties of gilts slaughtered at d 35 of gestation^a

Item	Supplemental B, mg/kg		SEM	P-value
	0	5		
Bone weight, g	495.7	455.5	19.2	0.16
Force at yield point, N	2,801	3,565	295	0.09
Bending moment at yield point, kN·mm	78.4	99.8	8.2	0.09
Yield stress, MPa	41.6	52.7	3.8	0.07
Modulus of elasticity at yield point, MPa	1153	987	113	0.33
Deformation, mm	2.9	4.2	0.47	0.08
Moment of inertia, mm ⁴	27,568	26,986	2,383	0.87

^aEach mean represents eight observations per treatment.

Table 6. Effect of boron supplementation on femur chemical composition and serum osteocalcin concentrations of gilts slaughtered at d 35 of gestation^a

Item	0	5	SEM	P-value
Percent lipid of femur	3.1	5.3	0.85	0.09
Fat-free ash percentage	69.6	69.6	0.39	0.98
Calcium, $\times 10^5$ mg/kg fat free ash	4.03	3.99	0.038	0.46
Phosphorus, $\times 10^5$ mg/kg fat free ash	1.80	1.75	0.028	0.25
Magnesium, mg/kg fat free ash	6,554.78	6,426.60	159.64	0.60
Zinc, mg/kg fat free ash	209.37	185.85	11.39	0.19
Copper, mg/kg fat free ash	3.01	3.13	0.26	0.76
Serum osteocalcin, ng/mL	252.79	288.27	15.33	0.13

^aEach mean represents eight observations per treatment.

B-supplemented gilts underwent more ($P = 0.08$) deformation at the yield point than femurs from control gilts.

Boron supplementation increased ($P = 0.09$) the lipid percentage of the femur (Table 6), which disagrees with previous work published by our laboratory (Armstrong et al., 2000); however, the animals used in these two studies were of different age and at two different physiological states. The fat-free ash percentage of the femur and the concentrations of Ca, P, Mg, Cu, and Zn were not affected ($P \geq 0.19$) by B supplementation. In contrast, B has increased bone ash percentage in poultry (Qin and Klandorf, 1991; Elliot and Edwards, 1992; Wilson and Ruszler, 1997).

Osteocalcin is the most abundant noncollagenous protein of bone, and its synthesis is partially regulated by 1,25-dihydroxycholecalciferol (Price and Baukol, 1980). Osteocalcin is produced by osteoblasts, and is also found in circulation. Serum osteocalcin originates from osteocalcin produced during cellular synthesis of new bone that did not bind to the mineral phase of bone (Price et al., 1981). Therefore, serum osteocalcin can provide a measure of osteoblast activity or bone remodeling (Price et al., 1981; Weinreb et al., 1990). Boron supplementation tended ($P = 0.13$) to increase serum osteocalcin concentrations (Table 6), which may indicate increased osteoblast activity.

Boron concentrations in the uterus, oviduct, ovary, and muscle were much higher ($P < 0.01$), and liver B concentrations were higher ($P = 0.06$) in gilts receiving supplemental B (Table 7). This is the first report in pigs

Table 7. Effect of dietary boron supplementation on tissue boron concentrations in gilts slaughtered at d 35 of gestation^a

Item	Supplemental B, mg/kg		SEM	P-value
	0	5		
Boron, ng/g				
Uterus	194	496	30	<0.01
Oviduct	155	342	24	<0.01
Ovary	103	302	24	<0.01
Embryo	452	1271	123	<0.01
Muscle	87	227	24	<0.01
Liver	209	308	33	0.06

^aEach mean represents seven observations per treatment.

demonstrating an increase in tissue B concentrations with B supplementation. Boron concentration of tissues, including muscle and liver, was increased by B supplementation in poultry (Wilson and Ruszler, 1996; 1998). In the present study, embryo B concentrations were increased ($P < 0.01$) nearly threefold by B supplementation.

Increasing dietary B decreased ($P \leq 0.04$) the concentration of Ca in the uterus, embryo, and oviduct (Table 8). Phosphorus concentrations of the uterus, ovary, and liver were decreased ($P < 0.01$) by supplemental B (Table 8). Previous studies have reported an interaction between dietary B and the metabolism of Ca and P (Nielsen et al., 1987; Hegsted et al., 1991). Although changes in mineral composition in response to dietary manipulation are too unspecific to provide a firm suggestion for the basis of changes, the increased Ca and P concentrations found in some soft tissues from gilts fed the low-B diet are consistent with the hypothesis that B has a role in cell membrane function, composition, or stability. Some phospholipids, sphingolipids, and cerebrosides contain chemical moieties that complex readily with B; these lipids are found in cell membranes. One of the richest sources of phospholipids and

Table 8. Effect of dietary boron supplementation on tissue concentrations of calcium and phosphorus in gilts slaughtered at d 35 of gestation^a

Item	Supplemental B, mg/kg		SEM	P-value
	0	5		
Calcium, $\mu\text{g/g}$				
Uterus	1,420	675	112	<0.01
Oviduct	399	364	10	0.04
Ovary	622	481	111	0.39
Embryo	2,745	1,739	158	<0.01
Muscle	116	120	4	0.46
Liver	110	109	4	0.93
Phosphorus, $\mu\text{g/g}$				
Uterus	7,477	6,967	98	<0.01
Oviduct	8,470	7,920	249	0.15
Ovary	11,307	9,404	149	<0.01
Embryo	12,018	11,921	412	0.87
Muscle	8,200	8,314	308	0.80
Liver	11,309	10,169	241	<0.01

^aEach mean represents seven observations per treatment.

cerebrosides is the brain. Boron deprivation has been found to increase the P concentration in brain cerebellum of rats (Dupre et al., 1994); the same rats also had increased liver P concentrations. Thus, the increased P found in the liver, ovary, and uterus in gilts fed the low-B diet in the present study perhaps reflects a change in cellular membrane composition, and this change might have an effect on the distribution of Ca to the uterus and ultimately to the embryo. The finding of increased Ca concentrations in the uterus, oviduct, and embryo should not be considered unique, because brain cortex Ca concentrations were increased by B deprivation in rats (Dupre et al., 1994), and B supplementation decreased the concentration of Ca (and P) in bone of chickens (Wilson and Ruszler, 1998). Changes in cell membrane function or Ca distribution also may have had some role in the finding of possible impaired reproductive function in gilts fed the low-B diet in the present study.

Implications

Long-term supplementation of boron to gilts increased the average pig weaning weight, tended to increase average pig birth weight, and increased boron concentrations of tissues, including the embryo. Increased weaning weight may yield improved production efficiency during the nursery phase. Increased boron concentration of the embryo indicates that boron is transferred across the placenta. In addition, boron increased the measures of intrinsic and extrinsic strength of the femur. Other reproductive characteristics were not affected statistically, yet there are numerical increases in a majority of these dependent variables that may have been significant if there had been a larger sample pool of gilts or if the basal diet had been lower in boron. Additional studies are warranted to accurately assess the effects of supplemental boron, with respect to reproductive characteristics and bone mechanical properties in pubertal gilts.

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